Serum copper and its relationship to clinical symptoms in rheumatoid arthritis

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SUMMARY Total serum copper and ultrafilterable copper levels in patients with rheumatoid arthritis were determined and related to articular index, erythrocyte sedimentation rate (ESR), and serum iron concentration. Relationships were found between serum copper and ESR, and between ultrafilterable copper and articular index. The relationship between serum copper and serum iron was found to be a drug-dependent.

The role of copper in rheumatoid arthritis has recently been of interest because of the abnormally high copper levels in the serum of patients with the disease (Bajpayee *et al.*, 1975; Scudder *et al.*, 1978), the greater anti-inflammatory activity of copper complexes of anti-inflammatory drugs (Sorensen, 1976), and a recent investigation of the persistent traditional use of copper bangles in the treatment of rheumatoid arthritis (Walker and Griffin, 1976). We report here our findings from a survey of over 100 patients with rheumatoid arthritis receiving different forms of therapy. In all cases the total serum copper concentration was estimated, and in a limited number the ultrafilterable copper level (using a cut-off of 1000 Dalton molecular weight) was determined by a newly developed method (Kamel *et al.*, 1978).

Patients and methods

One hundred and twenty patients (mean age $53.0 \pm \text{SEM } 4.8$ years), of whom 50 were male, were studied. All were suffering from classical or definite rheumatoid arthritis as defined by the criteria of the American Rheumatism Association (Ropes *et al.*, 1959). The mean duration of their disease was $9 \text{ years} \pm \text{SEM } 0.8$ years. All patients had radiological evidence of articular erosions, and in 70 patients the serological tests for IgM rheumatoid factor were significantly positive.

Total serum copper ultrafilterable copper were estimated by atomic absorption spectrometry using

carbon furnace atomisation (Kamel *et al.*, 1978). Pellican membranes (molecular weight cut-off, 1000) were used for ultrafiltration. Since the salt content depresses the copper signal, saline standards are required together with careful adjustment of the background corrector and, owing to the low copper concentration, considerable care to avoid contamination. Serum iron and total iron binding capacity were measured with ferrozene (Technicon AA II Method SF4—0025 FL 4), total protein by means of biuret (Technicon AA II Method N14a), and erythrocyte sedimentation rate by Westergren's method.

The articular index of joint tenderness (0 to 78 score units (Ritchie *et al.*, 1968) was measured in each patient by a physician and amanuensis.

Results

COPPER IN SERUM

The major copper containing constituent in serum is caeruloplasmin. Our results agree with those of Scudder *et al.* (1978) in that the total serum copper mean in patients with rheumatoid arthritis ($23.4 \pm 5.13 \mu\text{mol/l}$) was significantly higher than that of the controls ($16.06 \pm 3.93 \mu\text{mol/l}$) and that except in the few cases of very high serum copper (greater than $31 \mu\text{mol/l}$) the caeruloplasmin portion as measured by its oxidase activity accounted for about 90% of the total serum copper. Using a membrane with a molecular weight cut-off of 1000 we found a mean copper level in the ultrafiltrate of $0.853 \mu\text{mol/l}$ and a range from 0.047 to $1.458 \mu\text{mol/l}$. Small amounts of copper were possibly present in other protein fractions, but these were too close to the sensitivity limit of the methods used to give

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meaningful results. Occasional serum samples from females, both normal and rheumatoid, produced different distributions of copper, but these have not yet been fully elucidated and have not been included in any of the mean values cited.

COPPER VARIATIONS AND DRUG THERAPY

All the subjects in each group had been treated with the drug for at least 1 month prior to analysis of the sample for copper. The results in Table 1 suggest that there is a significant correlation between caeruloplasmin copper and drug therapy. For treatment with penicillamine and with steroids there is surprisingly good agreement with published results (Scudder *et al.*, 1978).

COPPER LEVELS AND CLINICAL PARAMETERS

In 100 patients the articular index measurements showed a slight but significant correlation with total serum copper and in 20 patients a much more significant correlation with ultrafilterable copper (Table 2).

In 90 patients a correlation was found between total serum copper and ESR, but in this case the correlation with ultrafilterable copper was much less significant.

A strong inverse correlation between serum iron and serum copper has been described in patients with rheumatoid arthritis (Scudder *et al.*, 1978). In a sample of 135 patients, of whom 50 were on levamisole therapy, we did not find any significant correlation (Table 2). However, if the levamisole treated patients were not included, a significant inverse relationship was found ($r = -0.348$, $P < 0.001$). Levamisole treated patients ($n = 50$) did not show a significant correlation with serum iron. The D-penicillamine treated group also failed to show a significant inverse correlation.

Table 1 Total serum copper with different drugs ($\mu\text{mol/l}$)

Steroids	23.46 ± 4.56	$n = 11$
D-penicillamine	20.70 ± 2.83	$n = 18$
Simple analgesics	25.98 ± 3.14	$n = 50$
Levamisole	22.67 ± 4.4	$n = 50$
Controls	16.06 ± 3.93	$n = 30$

Table 3 The correlation of high and low values of ESR, articular index, and iron concentration in serum with total serum copper levels. The dividing lines chosen are arbitrary. Copper levels are in $\mu\text{mol/ml}$

	Mean total serum copper	SD
Patients with ESR > 30 $n = 35$	25.07	± 4.73
" " ESR ≤ 30 $n = 50$	21.57	± 5.36
" " AI > 30 $n = 22$	25.86	± 4.41
" " AI ≤ 30 $n = 80$	22.55	± 5.20
" " Fe ≤ 13 $n = 98$	24.22	± 5.2
" " Fe > 13 $n = 58$	20.90	± 4.4

If arbitrary values of ESR, articular index, and serum iron are taken to mark a dividing line between 'severe' and 'not severe' rheumatoid arthritis, as shown in Table 3, the mean total serum copper levels were greater in the 'severe' category.

Discussion

Most serum copper is contained in caeruloplasmin with small amounts associated with other proteins and low molecular weight fractions. The biological activities, if any, of the latter fractions *in vivo* are not known, mainly because their concentrations are difficult to determine. Various methods of determining the copper level in these fractions have been employed, but all have drawbacks (Kamel *et al.*, 1978). In particular the determination of the copper in all these fractions by subtraction of the caeruloplasmin copper level from the total serum level seems to us to be unrealistic in view of the low copper levels and ligands associated with copper contamination from the chemicals and equipment used. Further, copper levels in caeruloplasmin can be determined either by calculation after analysis for the protein by immunoelectrophoresis, or as an oxidase, or by direct determination of the copper content. These are obviously closely related, but how precisely is as yet uncertain. In this study we analysed directly for ultrafiltrate and compared the levels obtained together with total serum copper levels to clinical parameters measured at the same

Table 2 The relationship of total copper and ultrafilterable copper levels in serum to erythrocyte sedimentation rate (ESR), articular index (AI), iron level, and % saturation of iron (SATN) in serum

	Articular index	ESR	Fe	SATN (%)
Total serum copper	$n = 100$ $r = 0.2266$ $P < 0.05$	$n = 90$ $r = 0.419$ $P < 0.001$	$n = 135$	$n = 50$ $r = -0.249$ $P < 0.1$
Ultrafilterable copper	$n = 20$ $r = 0.746$ $P < 0.001$	$n = 15$ NS	$n = 18$ NS	$n = 18$ $r = 0.4661$ $P = 0.05$

NS = Not significant.

time. Our results indicate that there is no simple relationship between total serum copper and ultrafilterable copper levels, and therefore that the ultrafiltrate is probably not in direct equilibrium with copper or albumin, though it seems likely. Thus the ultrafiltrate copper and the caeruloplasmin copper levels might be related differently to the usual clinical parameters. This is essentially what we found, in that the ultrafilterable copper showed a significant correlation with articular index whereas the total serum copper showed a much less significant correlation. The ultrafilterable copper showed no significant correlation with ESR measurements, whereas the total serum copper did show a significant correlation. Thus when talking of serum copper, at least 2 measurements should be considered—caeruloplasmin and low molecular weight compounds of copper. Any relationship between the ultrafilterable copper and the reported high urinary concentration of copper in rheumatoid arthritis (McMurray *et al.*, 1975) has not yet been established.

The other variable which has emerged is the effect of different drugs on total serum copper and ultrafilterable copper levels and on the clinical parameters articular index, serum iron, and ESR. Further work is necessary to assess the importance of the 2 copper estimations in different drug therapies.

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